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## Research paper

# Dry weight partitioning and hydraulic traits in young *Pinus taeda* trees fertilized with nitrogen and phosphorus in a subtropical area

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Plants of *Pinus taeda* L. from each of four families were fertilized with nitrogen (N), phosphorus (P) or N + P at planting. The H family had the highest growth in dry mass while the L family had the lowest growth. Measurements of plant hydraulic architecture traits were performed during the first year after planting. Stomatal conductance ( $g_s$ ), water potential at predawn ( $\Psi_{\text{predawn}}$ ) and at midday ( $\Psi_{\text{midday}}$ ), branch hydraulic conductivity ( $k_s$  and  $k_l$ ) and shoot hydraulic conductance ( $K$ ) were measured. One year after planting, dry weight partitioning of all aboveground organs was performed. Phosphorus fertilization increased growth in all four families, while N fertilization had a negative effect on growth. L family plants were more negatively affected than H family plants. This negative effect was not due to limitations in N or P uptake because plants from all the families and treatments had the same N and P concentration in the needles. Phosphorus fertilization changed some hydraulic parameters, but those changes did not affect growth. However, the negative effect of N can be explained by changes in hydraulic traits. L family plants had a high leaf dry weight per branch, which was increased by N fertilization. This change occurred together with a decrease in shoot conductance. Therefore, the reduction in  $g_s$  was not enough to avoid the drop in  $\Psi_{\text{midday}}$ . Consequently, stomatal closure and the deficient water status of the needles resulted in a reduction in growth. In H family plants, the increase in the number of needles per branch due to N fertilization was counteracted by a reduction in  $g_s$  and also by a reduction in tracheid lumen size and length. Because of these two changes,  $\Psi_{\text{midday}}$  did not drop and water availability in the needles was adequate for sustained growth. In conclusion, fertilization affects the hydraulic architecture of plants, and different families develop different strategies. Some of the hydraulic changes can explain the negative effect of N fertilization on growth.

**Keywords:** genetic variability, hydraulic conductivity, leaf water potential, nutrients, stomatal conductance, xylem anatomy.

## Introduction

Forest fertilization is a silvicultural practice used to improve site quality and productivity (Allen 1987). Fertilization is necessary to mitigate harvesting and soil tillage effects on site nutrient stocks and to maintain nutrient stability, site fertility and the sustainability of forest production (Nambiar 1997, Fox 2000, Goya et al. 2003). Increased nutrient availability influences stemwood production through effects on light interception, photosynthesis and carbon partitioning because light

interception is a function of leaf area (Cannell 1989). Nitrogen (N) and phosphorus (P) are the nutrients that most frequently limit plant growth (Aerts and Chapin 2000). Increases in growth due to P fertilization at establishment have been widely reported for *Pinus taeda* L. plantations in South America and the southern USA (Ibañez et al. 2004, Vogel et al. 2005, Fox et al. 2007, Everett and Palm-Leis 2009). However, decreases in growth associated with N fertilization at establishment have only been observed in subtropical zones that do not have a dry

season, like in northern Argentina and southern Brazil (Costa Muniz et al. 1975, Fernández et al. 1999, 2000a, Faustino et al. 2011). At these locations, *P. taeda* plantations achieve the highest productivity in the world, around  $30 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$  (Cubbage et al. 2007). The mechanisms that underlie the negative growth response to N fertilization are not well known, and although previous studies have proposed many hypotheses, the exact causes of this reduction in growth are not evaluated in any of them.

Changes in dry weight partitioning associated with improved N and P nutrition could lead to modifications in water use (Haynes and Gower 1995, Albaugh et al. 1998, Ewers et al. 1999, 2000) and in the hydraulic traits of trees (Bucci et al. 2006). The set of hydraulic characteristics of the conducting tissue of a plant which qualify and quantify the sap flux from roots to leaves are known as hydraulic architecture (Cruiziat et al. 2002). Examples of changes in hydraulic architecture related to nutrient availability have been documented. Fertilization increased leaf area but decreased both transpiration per unit of leaf area and sap flux per sapwood area in *Picea abies* (L.) Karst. (Phillips et al. 2001). In the same way, fertilization decreased stomatal conductance of the canopy in *P. taeda* (Ewers et al. 2001). Transpiration rate at plant level decreased with fertilization in three tropical trees (Cernusak et al. 2009), in *P. taeda* (Tyree et al. 2009a) and in *Quercus robur* L. (Welander and Ottosson 2000). Changes in stomatal conductance in fertilized plants have also been observed. For example, high N availability reduced stomatal conductance in cotton plants (Radin and Parker 1979), *Pinus contorta* Douglas ex Loudon (Amponsah et al. 2004), *Quercus prinus* L. (Kleiner et al. 1992) and in three species of Brazilian cerrado (Scholz et al. 2006). When other nutrients were also evaluated, stomatal conductance was lower in N-fertilized *Pinus pinaster* Aiton trees than in P-fertilized trees (Guehl et al. 1995). Nutrient availability can also change the capacity of wood to transport water (i.e., xylem conductivity). Nitrogen fertilization increased vessel diameter but decreased leaf specific conductivity and increased xylem vulnerability to cavitation in *Populus* (Harvey and van den Driessche 1997, 1999). If leaf specific water transport efficiency decreases without a concomitant change in resistance to cavitation, the risk of xylem embolism may increase and promote incomplete diurnal recharge of internal water storage, partial stomatal closure and inhibition of carbon assimilation (Bucci et al. 2006). In contrast, N fertilization did not change the  $k_1$  and  $k_s$  of *Eucalyptus grandis* W. Hill ex Maid. (Clearwater and Meinzer 2001). Changes in conductivity can be related to modifications in xylem cell morphology due to nutrient availability. For example, tracheid diameter in roots increased with N availability, but tracheid length was not affected in *Picea glauca* (Moench) Voss (Krasowski and Owens 1999). Tracheid lumen diameter tended to increase in

response to N availability in *Larix sibirica* Ledeb. (Yazaki et al. 2001) and in *P. abies* (Kostiainen et al. 2004), but in the latter species, tracheid lengths tended to be shorter. In all these cases, plants grew more with N fertilization; the opposite of our results. Faster growth caused by fertilization can be associated with a decrease in tracheid length because of rapidly dividing cambium cells (e.g., Ward et al. 2008). Changes in cell morphometry may or may not affect xylem conductivity, because sometimes the effects are counteracted and the final result is the same. For example, N fertilization increased conduit diameter at the root collar of *Eucalyptus pauciflora* Sieber ex Sprengel. However, this change was not associated with modifications in hydraulic conductivity (Atwell et al. 2009). In the same way, N fertilization increased vessel lumen diameter in *Populus* spp., but  $k_1$  decreased with this treatment due to the bigger leaf area produced by these plants (Harvey and van den Driessche 1997).

The changes in hydraulic architecture detailed above are species- and nutrient-dependent. Different *P. taeda* genotypes may employ different mechanisms to capture and distribute carbon when they are fertilized (Tyree et al. 2009a) and therefore may have different hydraulic architectures in response to increased soil nutrient availability.

The aim of this work was to examine the relationship between the effects of N and P fertilization on aboveground dry weight partitioning and on changes in hydraulic architecture in young trees from four *P. taeda* families, which can explain the negative effect on growth of N fertilization.

The hypotheses are: (i) N and P prompt different changes in plant hydraulic architecture. (ii) The depressive effect of N fertilization is not related with nutrient imbalances, but it is related with changes in some hydraulic traits that affect negatively the water status of the plants. (iii) The changes in hydraulic architecture are related with different anatomy and morphology of N-fertilized plants. (iv) Families have different hydraulic architecture, therefore N fertilization will affect them to dissimilar extents, and consequently the effect of fertilization on growth will be diverse.

## Materials and methods

### Site conditions, plant material and treatments

The experiment was established in Montecarlo, Misiones, Argentina ( $26^{\circ}30'S$ ,  $54^{\circ}40'W$ ). Mean annual rainfall and temperature in the area are 2000 mm, evenly distributed throughout the year, and  $20^{\circ}C$ , respectively. The soil is clay, a red and deep ultisol. It has 2.29% of organic carbon, 0.21% of total nitrogen, 2.20 ppm of extractable phosphorus. The soil pH is 5.05 and the cation exchange capacity (CEC) is  $9.00 \text{ cmol kg}^{-1}$ . The soil contains 1.45% of sand, 32.20% of silt and 66.35% of clay. Soil chemical analyses were performed using the following methods: organic carbon by dry combustion with an

automatic analyzer (CR12-LECO), total N by the semimicro Kjeldahl method, P by the Bray–Kurtz method, pH by the potentiometric method (1 : 2.5) and CEC by the Polemio–Rhoades method. Texture components were evaluated using the Robinson pipette method. These analyses were performed by the LANAIS N15 laboratory, which belongs to the CONICET–UNS (Consejo Nacional de Investigaciones Científicas y Técnicas – Universidad Nacional del Sur) in Argentina.

Four fast-growing families of *Pinus taeda* L. were used, provided by the plant breeding and genetics program at INTA Montecarlo, Argentina. The selected families have different growth rates: H and IH have higher growth rates than the L and IL families. All the families have similar basic wood densities as adult trees.

Six-month-old seedlings were planted in September 2009 using 0.8 m × 0.8 m spacing to minimize soil and light spatial heterogeneity. The experiment concluded in November 2010. Each plot had 16 plants: 4 plants belonged to each family (4 subplots per plot). A 2 × 2 × 4 factorial design was used and included the following treatments: nitrogen (–N or +N), phosphorus (–P or +P) and family (H, IH, IL or L) of *P. taeda*. Therefore, each family was fertilized with N, P, N and P, or not fertilized. Three replicates were installed, for a total of 12 plots and 48 subplots. Fertilizer was applied immediately after planting. The fertilizers were put in two holes at 10 cm from the plant collar. The holes were covered with soil. Nitrogen was applied as 0 g (–N) and 100 g (+N) of urea (46-0-0) per plant (0 and 46 g of N), and P as 0 g (–P) and 200 g (+P) of calcium super phosphate (0-48-0) per plant (0 and 96 g of PO<sub>5</sub>). The doses chosen were evaluated in previous experiments. In these experiments, P increased growth while N has a negative effect at the above concentrations (Fernández et al. 2000a, Faustino et al. 2011).

Temperature and relative humidity were continuously recorded with a meteorological station (DAVIS–GroWeather). Rainfall, temperature and air saturation deficit during the period of the experiment are shown in Figure 1. Monthly average temperatures for each month were similar to historical averages for the zone, except for October and November 2010, which were on average 3 °C lower than historical average temperatures. Rainfall during the first spring (September–December 2009) was around 100 mm higher than the historical precipitation for the zone. This trend continued throughout summer. Accumulated rainfall during the autumn was similar to historical records, while in winter (July 2010) and during the second spring, precipitation was nearly 100 mm lower than historical data.

#### Growth measurements and foliar nutrient concentration

At the end of the experiment, the aboveground portion of each plant was cut at ground level and separated into needles, branches and main stem to estimate dry weight for each

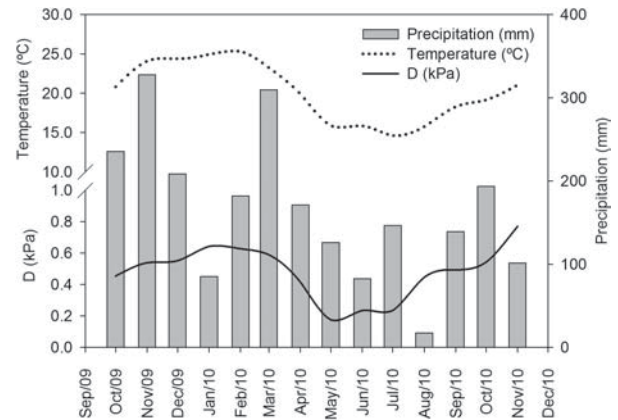


Figure 1. Monthly average temperature (°C), monthly average air saturation deficit (*D*) and accumulated monthly precipitation (mm) observed during the experiment.

compartment and calculate the allometric relationships. Samples were oven dried at 65 ± 5 °C and weighed to the nearest 0.1 g.

To determine N and P concentration, pooled needle samples (from the four trees in each subplot) were taken from each of the 48 subplots. Nitrogen concentration was determined using the semi-micro Kjeldahl method and P concentration by induced plasma emission spectroscopy. Foliar N and P contents were calculated as the product of nutrient concentration and the total needle dry weight.

#### Leaf water potential and stomatal conductance

Leaf water potential during the early morning ( $\Psi_{\text{predawn}}$ ; MPa) and at midday ( $\Psi_{\text{midday}}$ ; MPa) and stomatal conductance ( $g_s$ ; mmol H<sub>2</sub>O m<sup>–2</sup> s<sup>–1</sup>) were measured in March, June and August 2010 (2 sunny days in each month). The data from the six measurement days were averaged since the values were similar for each treatment. Air saturation deficit (*D*) ranged between 0.03 and 2.02 kPa. To take into account the possible effects of *D* on  $g_s$ ,  $\Psi_{\text{midday}}$  and  $\Psi_{\text{predawn}}$ , the data were analyzed using *D* as a covariate. The measurements were performed using plants from the H and L families in the unfertilized (–N – P), fertilized with N (+N – P) and fertilized with P (–N + P) treatment groups. Leaf water potential was measured with a pressure chamber on fully expanded sun-exposed fascicles. At least five plants per treatment were chosen haphazardly for the measurement. Each sample was cut and immediately put in a plastic bag and then measured ~1 min after cutting. Stomatal conductance was measured four times a day, in at least eight plants per treatment, using a steady-state porometer (SC-1, Decagon Devices Inc., WA, USA). Only fully expanded, sun-exposed fascicles were measured. Each measurement was made in a group of fascicles enough to fill the chamber.

#### Shoot hydraulic conductance and branch conductivity

In November 2010, the hydraulic conductance of the shoots (branches with needles) (*K*; g MPa<sup>–1</sup> s<sup>–1</sup>) and the hydraulic

conductivity ( $k_h$ ;  $\text{g m MPa}^{-1} \text{ s}^{-1}$ ) of the branch xylem (branches without needles) were determined by the low-pressure steady-state flow meter (SSFM) method. This method uses the drop in pressure across a tube of known resistance, together with the pressure at the stem fitting, to measure the flow rate into the stem segment or branch with needles (Brodribb and Feild 2000, Zwieniecki et al. 2000). The measurements were performed using plants from the H and L families in the unfertilized ( $-N - P$ ), fertilized with N ( $+N - P$ ) and fertilized with P ( $-N + P$ ) treatment groups. One branch from each of five plants per treatment was chosen haphazardly for measurement. The samples were collected during the early morning from the middle of each tree crown. All branches had the same cardinal orientation and similar lengths and diameters. The samples were put in plastic bags, cut, immediately submerged in a water container and taken to the laboratory. Prior to connecting each branch to the equipment, the distal end of the branch was cut underwater. Hydraulic resistance of the shoots was measured when the flow was stable. These measurements represent the morning baseline resistance, when the likelihood of embolism is lower. First, the  $K$  of the shoots was measured. Then, after cutting off the portion of the branch with needles, the hydraulic conductivity of the branch ( $k_h$ ) was measured, such that only the basal portion of the branch (50 mm in length) was used. To take into account the effect of size on  $K$ , the data were analyzed using the leaf area of each shoot as a covariate. Branch specific hydraulic conductivity ( $k_s$ ;  $\text{g MPa}^{-1} \text{ s}^{-1} \text{ m}^{-1}$ ) and leaf specific hydraulic conductivity of the branch ( $k_l$ ;  $\text{g MPa}^{-1} \text{ s}^{-1} \text{ m}^{-1}$ ) were calculated from  $k_h$  divided by the xylem cross-sectional area of the branch or leaf area supported by the branch, respectively.

### Light microscopy observations of xylem

Transverse sections from each branch that was used to measure hydraulic conductivity were analyzed ( $n = 5$  branches per treatment). Branch cross sections (25  $\mu\text{m}$  thick) were obtained with a sliding microtome from two complete radii (from pith to cambium), and then stained for 5 min with safranin (5%) and mounted in Entellan® rapid mounting medium (Merck, Darmstadt, Germany) for microscopy. Stained sections were photographed with a digital camera (Olympus DP71, Tokyo, Japan) mounted on a microscope (Olympus BX50, Tokyo, Japan) using the  $\times 40$  objectives. Captured images were analyzed using image analysis software (ImagePro Plus, v 6.3, Media Cybernetics, Rockville, MD, USA) for the following parameters: tracheid lumen diameter ( $n = 130$  per sample,  $n = 780\text{--}1300$  per treatment) and tracheid density (number per  $\text{m}^2$ ) ( $n = 5$  to 10 captured images per sample, image area = 31,202  $\mu\text{m}^2$ ). To measure tracheid lengths, macerations of bark-free xylem from all the branches were made in a 1 : 1 solution of acetic acid and hydrogen peroxide (20%)

(Franklin 1945). At least 150 tracheids per sample were measured in photographs taken on the microscope described previously.

### Statistical analysis

Data were analyzed using analysis of variance (ANOVA). For aboveground dry weight compartments and dry weight ratios, family (H, IH, IL and L), N ( $-N$  and  $+N$ ) and P ( $-P$  and  $+P$ ) were used as main factors. For the leaf N and P concentrations and contents, the family factor included only two levels (H and L). For  $g_s$ ,  $\Psi_{\text{predawn}}$ ,  $\Psi_{\text{midday}}$ ,  $K$ ,  $k_s$ ,  $k_l$  and xylem characteristics, the main factors were family (H and L) and fertilization ( $-N - P$ ,  $-N + P$  and  $+N - P$ ). To evaluate  $g_s$ ,  $\Psi_{\text{predawn}}$  and  $\Psi_{\text{midday}}$ , the analysis was performed using  $D$  as a covariate (analysis of covariance (ANCOVA)). For each hydraulic parameter,  $D$  was calculated for the same time of day as the parameter was measured. For the  $K$  analysis, the leaf area of the shoot was used as covariate. In all cases, when the ANOVA was significant ( $P \leq 0.05$ ), the means were compared using Fisher's least significant difference test (LSD) test ( $\alpha = 0.05$ ).

## Results

### Aboveground dry weight partitioning and foliar nutrition

All aboveground dry weight compartments were significantly affected by N, P and family, but the interactions between the factors were not significant (Table 1). Considering all four families together, N significantly depressed growth and P increased growth of all aboveground compartments and total aboveground dry weight. On the other hand, there were differences in dry weight accumulation between families. Plants from families with higher growth rates were significantly larger than plants from families with lower growth rates. Moreover, plants in the L family were smaller than those in the IL family (Table 1). Although there were no significant interactions between fertilization and family in total aboveground dry weight, plants in the L family were more negatively affected by N and less favored by P than those in the H family. Also, plants in the H family were not as negatively affected by N (Figure 2).

In addition, aboveground dry weight partitioning was significantly affected by the main factors N, P and family (Table 1). L family dry weight partitioning was different compared with the other families. Plants in the L family had a higher leaf : total aboveground dry weight ratio and lower branches : total aboveground dry weight ratio than plants from the other families. This means that plants in the L family had a higher leaf dry weight per branch (Table 1). For all families, N significantly increased the leaf : total aboveground dry weight ratio, decreased branch : total aboveground dry weight ratio and, consequently, increased the leaf : branch dry weight ratio (Table 1). For all families, P significantly reduced the leaf : total aboveground dry weight and leaf : branch dry weight ratios (Table 1).



Table 1. Means and *P* values from the ANOVA for dry weight compartments (g) and dry weight ratios, considering nitrogen (N), phosphorus (P) and family (F) as main factors. Means followed by different letters in each column and for each factor are significantly different (Fisher's LSD). Bold type highlights *P*-values significant at the 0.05 level.

Factor	Level	Dry weight				Ratios		
		Leaf	Branch	Main Stem	Total	Leaf : Total	Branch : Total	Leaf : Branch
N	–N	145.5 b	69.7 b	101.2 b	316.3 b	0.48 a	0.20 b	2.67 a
	+N	114.9 a	47.5 a	79.2 a	241.5 a	0.50 b	0.18 a	3.17 b
	<i>P</i>	<b>0.033</b>	<b>0.043</b>	<b>0.037</b>	<b>0.024</b>	<b>0.050</b>	<b>0.026</b>	<b>0.027</b>
P	–P	92.9 a	44.4 a	58.0 a	195.3 a	0.51 b	0.19	3.24 b
	+P	163.5 b	70.8 b	119.0 b	353.2 b	0.48 a	0.18	2.64 a
	<i>P</i>	<b>&lt;0.001</b>	<b>0.010</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.008</b>	0.498	<b>0.004</b>
F	H	171.3 c	76.5 bc	124.7 c	372.4 c	0.47 b	0.19 b	2.75 b
	IH	159.6 c	81.8 c	114.4 c	355.8 c	0.48 b	0.21 b	2.49 b
	IL	114.2 b	52.8 b	80.6 b	247.6 b	0.48 b	0.20 b	2.56 b
	L	74.0 a	21.5 a	40.1 a	135.5 a	0.54 a	0.15 a	3.89 a
	<i>P</i>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
N × P	<i>P</i>	0.488	0.523	0.770	0.677	0.732	0.183	0.211
N × F	<i>P</i>	0.220	0.477	0.318	0.312	0.292	0.625	0.456
P × F	<i>P</i>	0.083	0.690	0.060	0.076	0.785	0.416	0.356
N × P × F	<i>P</i>	0.910	0.608	0.925	0.944	0.758	0.591	0.981

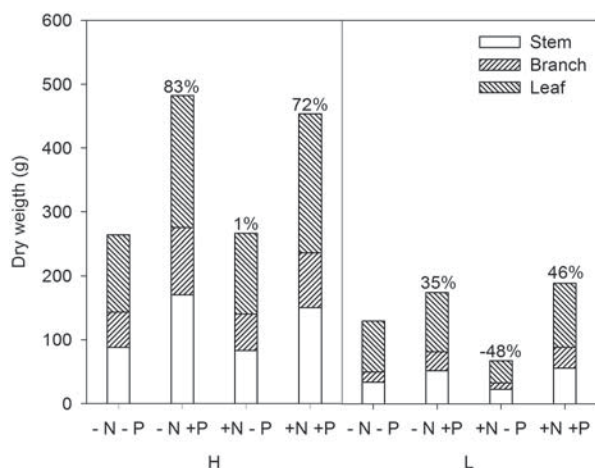


Figure 2. Aboveground dry weight partitioning and total aboveground dry weight of the H (high growth rate) and L (low growth rate) families, with N (+N), without N (–N), with P (+P) and without P (–P). Numbers over the bars indicate the percentage of change relative to unfertilized plants (–N – P) for each family.

Foliar N and P concentrations were not significantly affected by the main factors N, P and family (Table 2). Differences in foliar N and P content between treatments were directly related to differences in foliar dry weight (data not shown). Therefore, total nutrients per tree bole decreased in the N treatment because the total leaf dry weight decreased. On the other hand, N retained in the needles was higher in +P plants. Foliar N content : total aboveground dry weight ratio was significantly affected by the N and family factors (Table 2). It was higher in +N and L family plants than in –N and H family plants.

#### Leaf water potential and stomatal conductance

Pre-dawn leaf water potential ( $\Psi_{\text{pre-dawn}}$ ) was not different for any family or treatment and was not influenced by *D*. Midday

Table 2. Means and *P* values from the ANOVA for N and P foliar concentration ([N] and [P]) ( $\text{mg g}^{-1}$ ) and N foliar content by total aboveground dry weight ( $N_{\text{leaf}} : DW_{\text{total}}$ ) ( $\text{mg g}^{-1}$ ), considering nitrogen (N), phosphorus (P) and family (F) as main factors. If  $P < 0.05$ , means followed by different letters in each column and for each factor were considered to be significantly different (Fisher's LSD). Bold type highlights *P*-values significant at the 0.05 level.

Factor	Level	[N]	[P]	$N_{\text{leaf}} : DW_{\text{total}}$
N	–N	17.15	1.45	8.45 a
	+N	18.07	1.25	9.31 b
	<i>P</i>	0.170	0.119	<b>0.003</b>
P	–P	17.47	1.29	9.11
	+P	17.80	1.38	8.75
	<i>P</i>	0.492	0.424	0.436
F	H	17.89	1.29	8.51 a
	L	17.41	1.39	9.31 b
	<i>P</i>	0.587	0.502	<b>0.002</b>
N × P	<i>P</i>	0.320	0.138	0.631
N × F	<i>P</i>	0.285	0.552	0.811
P × F	<i>P</i>	0.249	0.175	0.213
N × P × F	<i>P</i>	0.267	0.230	0.052

leaf water potential ( $\Psi_{\text{midday}}$ ) covaried strongly with *D* (Table 3). There was also a significant interaction between family and fertilization for the  $\Psi_{\text{midday}}$  analysis. For the L family,  $\Psi_{\text{midday}}$  in +N – P plants was significantly lower than in the other fertilization treatments, while it was similar among all the treatments in the H family (Table 3 and Figure 3).

Daily average  $g_s$  also covaried strongly with *D*. Individually, family and fertilization significantly affected this variable, but the interaction between these factors was not significant (Table 3). Average  $g_s$  was significantly lower in +N – P than in the other fertilization treatments (Figure 4b). For all treatments, plants in the H family had significantly higher  $g_s$  than those in the L family (Figure 4a).

Table 3. Summary of the  $P$  values from the ANCOVA of stomatal conductance ( $g_s$ ) ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), pre-dawn leaf water potential ( $\Psi_{\text{predawn}}$ ) (MPa) and midday leaf water potential ( $\Psi_{\text{midday}}$ ) (MPa), considering fertilization treatment and family as main factors and air saturation deficit ( $D$ ) (kPa) as the covariate. Bold type highlights  $P$ -values significant at the 0.05 level.

Parameter	Main effects			Two-way interactions
	$D$	Fertilization treatment	Family	Fertilization treatment $\times$ Family
$g_s$	<b>&lt;0.001</b>	<b>0.003</b>	<b>0.026</b>	0.296
$\Psi_{\text{predawn}}$	0.649	0.085	0.088	0.771
$\Psi_{\text{midday}}$	<b>&lt;0.001</b>	<b>0.007</b>	0.203	<b>0.002</b>

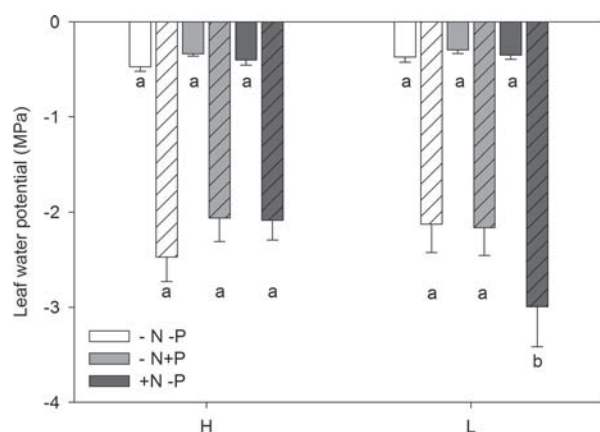


Figure 3. Predawn ( $\Psi_{\text{predawn}}$ ) and midday ( $\Psi_{\text{midday}}$ ) leaf water potential (MPa). Plain bars represent  $\Psi_{\text{predawn}}$  measurements and striped bars represent  $\Psi_{\text{midday}}$  measurements.  $P$  values for the main factors (fertilization treatment and family) are shown in Table 3. Different letters denote significant differences (Fisher's LSD) for each time of the day. Bars indicate standard errors.

### Shoot hydraulic conductance, branch hydraulic conductivity and xylem anatomy

There was a significant interaction between family and fertilization in shoot hydraulic conductance ( $K$ ) ( $P = 0.022$ ). There was no covariance between  $K$  and the leaf area of the shoot ( $P = 0.260$ ). There were no differences between  $-N - P$  and  $-N + P$  for any of the families. For plants in the L family,  $K$  was lower in  $+N - P$  plants than in the other treatments. In contrast, for plants in the H family,  $K$  did not differ among the three treatments (Figure 5a).

Branch  $k_s$  and  $k_l$  were not significantly affected by family or fertilization or their interaction (Table 4 and Figure 5b). There was a significant interaction between family and fertilization for tracheid lumen diameter, tracheid density and tracheid length (Table 4). For the H family, the tracheid lumens of  $+N - P$  and  $-N + P$  plants were smaller than those in the  $-N - P$  treatment (Figure 6a). Consistently, tracheid density was lower in  $-N - P$  plants than in the fertilized ones (Figure 6b). In this family, tracheid length was shorter in  $+N - P$  plants than in  $-N + P$

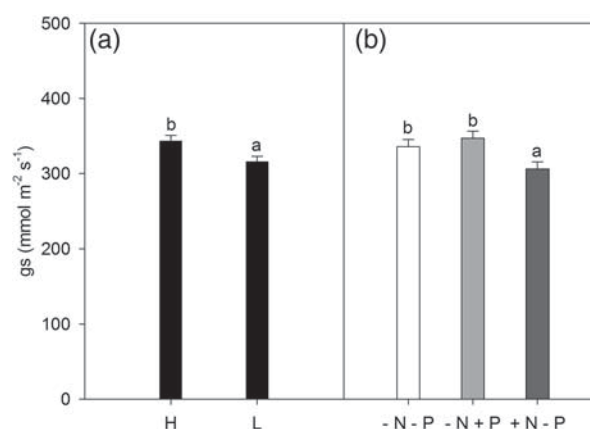


Figure 4. Daily average stomatal conductance ( $g_s$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) for the significant factors: (a) family and (b) fertilization treatment.  $P$  values for the main factors (fertilization treatment and family) are shown in Table 3. Different letters denote significant differences (Fisher's LSD). Bars indicate standard errors.

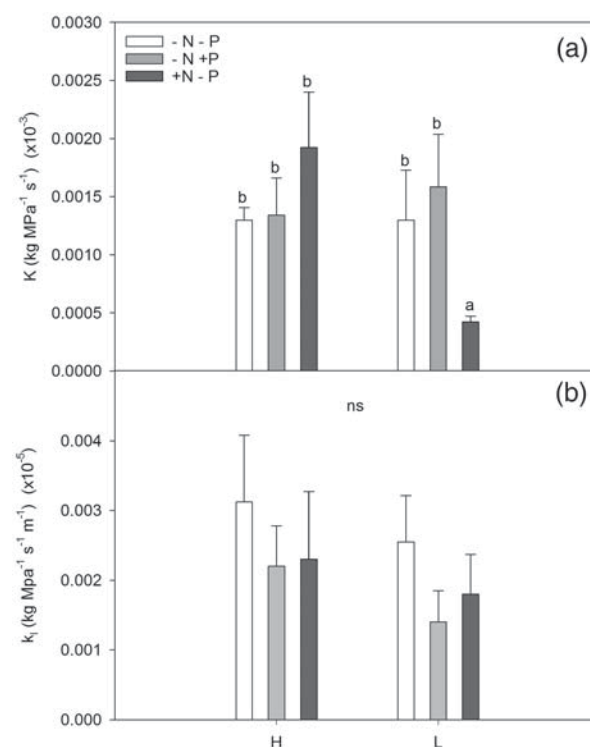


Figure 5. (a) Shoot hydraulic conductance ( $K$ ;  $\text{g MPa}^{-1} \text{s}^{-1}$ ). The interaction term between family and fertilization treatment was significant ( $P = 0.022$ ). Different letters indicate significant differences (Fisher's LSD). (b) Leaf specific hydraulic conductivity ( $k_l$ ) ( $\text{g s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$ ) of the branch. None of the main effects or their interactions were significant (Table 4). Bars indicate standard errors.

plants, and both treatments had shorter tracheids than  $-N - P$  plants (Figure 6c). For the L family, lumen diameter was not different between fertilization treatments (Figure 6a). However,  $-N + P$  plants had lower tracheid densities than  $-N - P$  plants (Figure 6b). In this family, only those in the  $-N + P$  treatment

Table 4. Summary of *P* values from the ANOVA of branch specific hydraulic conductivity ( $k_s$ ) ( $\text{g s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$ ), leaf specific hydraulic conductivity of the branch ( $k_l$ ) ( $\text{g s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$ ), tracheid lumen diameter ( $\mu\text{m}$ ), tracheid length ( $\mu\text{m}$ ) and tracheid density ( $n \mu\text{m}^{-2}$ ), considering fertilization treatment and family as main factors. Bold type highlights *P*-values significant at the 0.05 level.

Parameter	Main effects		Two-way interactions
	Fertilization treatment	Family	Fertilization treatment $\times$ Family
$k_s$	0.557	0.224	0.443
$k_l$	0.059	0.203	0.840
Tracheid lumen diameter	<b>0.026</b>	<b>0.001</b>	<b>0.001</b>
Tracheid length	<b>&lt;0.001</b>	<b>&lt;0.011</b>	<b>&lt;0.001</b>
Tracheid density	<b>0.038</b>	0.070	<b>&lt;0.001</b>

showed a decrease in tracheid length with respect to the  $-N - P$  treatment (Figure 6c).

## Discussion

### Growth, dry weight partitioning and nutrient concentration

Phosphorus fertilization increased growth in all the families, while N fertilization had a negative effect (Table 1), as had been observed in previous studies on *P. taeda* plantations in subtropical areas of South America (Costa Muniz et al. 1975, Fernández et al. 1999, 2000a, Faustino et al. 2011). These previous studies prompted us to investigate the physiological causes of the negative effect that N fertilization has on growth. It is interesting to note that, although no interaction between fertilization and family was observed, low growth rate families were more negatively affected by N fertilization and had a lower response to P fertilization than fast growth rate families. To investigate the possible reasons for these differences, we compared H and L families because of their lack of response and negative responses to N fertilization, respectively (Figure 2).

The first question we addressed was whether the fertilizers were taken up by the plants. Nitrogen and P foliar concentration did not differ between treatments and the values were similar to the optimal values and N : P ratios ( $>10.0$ ) reported for *P. taeda* (Allen 1987, Needham et al. 1990, Jones Benton 1993). Furthermore, foliar concentrations were similar to those found in other studies with young *P. taeda* plantations in the same area (Fernández et al. 2000a, 2000b, Goya et al. 2010). Consequently, the differences in growth cannot be explained by problems in nutrient uptake. As needle P concentration was similar in all the treatments, but P fertilization increased growth, P uptake was just enough to sustain growth and no P luxury consumption or dilution occurred (Birchler et al. 1997). Therefore, P fertilized plants had higher P availability in the soil, they took up

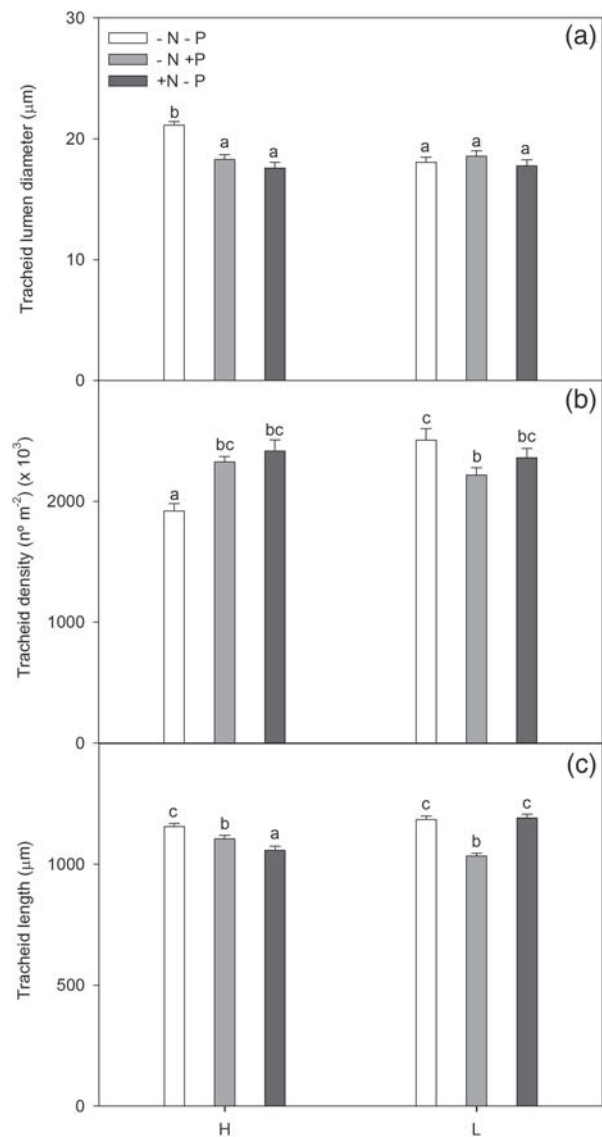


Figure 6. (a) Tracheid lumen diameter ( $\mu\text{m}$ ), (b) tracheid density ( $n \mu\text{m}^{-2}$ ) and (c) tracheid length ( $\mu\text{m}$ ). *P* values for the main factors are shown in Table 4. Different letters denote significant differences (Fisher's LSD). Bars indicate standard errors.

more P and, consequently, grew more. Similarly, needle N concentration did not differ among the fertilization treatments. Nitrogen-fertilized plants in the L family grew less than the unfertilized plants. Therefore, despite the higher availability of N in the soil, the plants did not take up more N. Instead, they took up this nutrient in proportion to their size. Therefore, in N-fertilized plants, neither dilution nor luxury consumption was observed. These results suggest that the problem caused by N fertilization is not related to a direct nutritional effect (e.g., toxicity). No changes have been found in soil pH due to urea fertilization in this type of soil (L. I. Faustino, unpublished data).

An increase in leaf area due to high nutrient availability is a typical response in this species (Green and Mitchell 1992, Green et al. 1994, Albaugh et al. 1998, 2004, Coyle et al. 2008, Tyree

et al. 2009b). Also, increases in the amount of foliage carried by each branch are induced by N fertilization (Gillespie et al. 1994) (Table 1). Despite the lack of changes in foliar N and P concentration due to fertilization (Table 2), nutrient content did change. Increases in the number of needles and in plant size mean that the total nutrient content in the leaves was higher in the family with a high growth rate (H). Plants in the H family used these nutrients more efficiently because, with the same foliar N and P concentration, H family plants grew more than those in the L family. This resulted in a lower amount of N retained in the needles for each gram of dry weight in the H family compared with the L family (Table 2). In both families, P fertilization increased dry weight but plants had the same needle nutrient concentration. On the other hand, while foliar N concentration was the same in N-fertilized and unfertilized plants, N-fertilized plants had lower total dry weight and they accumulated proportionally more dry weight in their leaves (Tables 1 and 2). Therefore, the amount of N retained in leaves per gram of total aboveground dry weight produced was higher in N-fertilized plants (Table 2). This means that N-fertilized plants could not use the proportionally higher N accumulated in their leaves to sustain higher growth. This suggests that N-fertilized plants are less efficient in converting the N retained in the leaves into aboveground growth. Although root growth was not considered in this experiment, in general, allocation to roots is lower in N-fertilized plants (Ewers et al. 1999, Samuelson et al. 2008a, Bakker et al. 2009). If this was the case in this experiment, the negative effect of N in growth would be even more severe.

Based on these data, we conclude that there does not appear to be N deficiency or excess in the soil, either in fertilized or unfertilized plants. Therefore, the negative effect of N fertilization on plant growth is likely caused by another factor. Because of this, changes in the hydraulic architecture (i.e., the capacity for water transport or use) were analyzed. Moreover, the different responses observed in plants in the H and L families imply that the families can employ different nutrient and water use strategies.

### Hydraulic changes due to fertilization in different families

Previous studies in 8-year-old *P. taeda* plantations have observed  $\Psi_{\text{midday}}$  values ranging from  $-1$  to  $-1.6$  MPa (Ewers et al. 2000, Samuelson et al. 2008c). These values are higher than our results. This suggests that, in our study, needles had low water availability, possibly a result of reduced root system development and low soil hydraulic conductivity. The latter is typical of soils with contractile clays, like those used in our experiment, when water availability in the soil is low. Although rainfall is enough to sustain the high growth of this species, water availability in the soil can drop during short periods due to high atmospheric demand from evapotranspiration (Figure 1). Therefore, low  $\Psi_{\text{midday}}$  can be a sign of water restriction to leaves, which is likely to be related to low water transport capacity in the soil or plant, and not to insufficient rainfall.

Phosphorus fertilization changed hydraulic architecture in aspects related to changes in plant size, like the higher aboveground dry mass observed here and height and collar diameter (Faustino et al. 2012). Larger plants may have longer water pathways (Midgley 2003), larger total leaf areas and probably release a larger amount of water into the atmosphere per plant. On the other hand, N fertilization changed size-independent aspects of the hydraulic architecture. Plants in the L family were the most negatively affected by N fertilization, with respect to growth, and they also had the lowest  $\Psi_{\text{midday}}$  (Figure 3). Previous studies have reported that water potential in leaves is affected by fertilization (Stoneman et al. 1996, Bucci et al. 2006, Lovelock et al. 2006). In *P. taeda*, Samuelson et al. (2008b) found that fertilization increases  $\Psi_{\text{midday}}$  in comparison with unfertilized plants. On the other hand, Ewers et al. (2000) found that  $\Psi_{\text{midday}}$  did not vary with fertilization.

Together with a decrease in  $\Psi_{\text{midday}}$ , N-fertilized plants in the L family had the lowest daytime  $g_s$  (Figure 4). Similar to our findings, in several other studies fertilization with N resulted in lower  $g_s$  in *P. taeda* seedlings (Samuelson 2000, Munger et al. 2003, Tyree et al. 2009a). However, still other studies showed unclear modifications in  $g_s$  following fertilization (Murthy et al. 1996).

As  $\Psi_{\text{predawn}}$  was high and similar in all treatments, it is reasonable to think that the cause of the lower  $g_s$  and  $\Psi_{\text{midday}}$  in N-fertilized plants from the L family was associated with a lower water supply to the leaves. Therefore, partial stomatal closure was a consequence of the drop in leaf  $\Psi$  because water delivery to the leaves was not enough to counteract water losses from transpiration. This hypothesis is possible since the dry weight partitioning of plants in the L family was different than plants in the H family; each branch of L family plants supported more leaves per branch compared with plants in the H family and N fertilization also increased the quantity of leaves supported by each branch in both families (Table 1). The same pattern was observed in the ratio between leaf area to xylem area (LA : XA) of the branches used to measure conductance (data not shown). Therefore, in L family plants fertilized with N, the xylem of each branch was subject to a higher demand for water relative to the other treatments. Moreover, there was a marked decrease in the hydraulic conductance ( $K$ ) of the shoots in N-fertilized plants compared with P-fertilized or unfertilized plants (Figure 5). On the other hand, P fertilization decreased the proportion of total leaf area sustained by each branch (Table 1), did not change  $K$ , and consequently  $g_s$  and leaf  $\Psi$  did not decrease (Figures 3–5).

The decrease in conductance can be due to different xylem architecture (e.g., narrower or shorter tracheids). Nevertheless, plants in the L family fertilized with N did not show changes in tracheid lumen diameter, density or length (Figure 6), or in  $k_s$  or  $k_l$  (i.e., the capacity of the branches to transport water) with respect to non-fertilized plants (Table 4 and Figure 5b). Therefore, the lower  $K$  could be due to changes in needle



anatomy or fascicle junctions with branches or from an increased number of permanent embolisms. During measurement, the branch and needles were hydrated, but permanent embolisms in the xylem were not removed. Therefore, the hydraulic conductance obtained was representative of the real conductive capacity of plants growing in field conditions.

Changes in dry weight partitioning, together with the decrease in  $K$ , can explain why  $g_s$  was lower and  $\Psi_{\text{midday}}$  drops in L family plants fertilized with N. In another study of *P. taeda*, needles constituted around 75% of the aboveground hydraulic resistance to water flow (Domec et al. 2009b). Therefore, one possible cause of the lower growth in plants from the L family could be that the lower  $g_s$ , together with the same photosynthetic capacity, would result in lower carbon fixation and, consequently, lower growth. Presumably, photosynthetic capacity was similar in all the families and treatments because N needle concentration was similar and these parameters are correlated (Gough et al. 2004). Nevertheless, as the response of carbon assimilation to  $g_s$  is not linear and the L family has lower  $g_s$  in all treatments, it is likely that the effect of lower  $g_s$  on assimilation in L family is disproportionate to that in H family. In addition, the lower water potential in the leaves could have contributed to diminished cell turgor, such that growth could also have been limited by lower cell expansion in growing tissues.

In the case of the H family, N fertilization also decreased  $g_s$  to values similar to those exhibited in the L family. However, in the H family, stomatal closure was more efficient because water potential did not drop (Figure 3). The latter is probably related to the lower dry weight of leaves per branch observed in the H family (Table 1) and also because shoot hydraulic conductance tended to be higher than in the other treatments (Figure 5). Furthermore,  $k_s$  and  $k_l$  was similar in fertilized and unfertilized plants, which is probably related to the ability of this family to reduce tracheid lumen diameter and length, but increase tracheid number following fertilization (Figure 6). Shorter tracheids with smaller diameters should impose higher resistance to water movement (Ewers et al. 1999). However, in the fertilized plants of this family, reduction in tracheid size was counteracted by an increase in their number; therefore, the conductivity did not change. Smaller lumens reduce water flow but also reduce the risk of embolism (Tyree and Ewers 1991, Bucci et al. 2006). The risk of embolism was probably reduced due to the smaller tracheid diameter in N-fertilized plants. Thus, in H family plants, fertilization with N increased the number of needles per branch, but did not significantly reduce xylem conductivity. Embolisms were probably less frequent, such that delivery of water to the needles was ensured and fertilization did not have such a negative effect as was observed in L family plants. The measurements of branch conductance in our study were taken at water potentials reflecting the maximum field hydration; thus they may not represent the

effect of reversible embolisms that occur on a diurnal basis. As leaf hydraulic conductance shows very large fluctuations on a diurnal basis in this species (Domec et al. 2009a), further investigations should be done into the mechanisms underlying the hydraulic effects of N fertilization because they may depend on this diurnal cycle of reversible embolisms.

## Conclusion

This work suggests a possible explanation for the negative effect of N fertilization on growth in young *P. taeda* plants. Problems with N or P uptake were rejected as the cause of this effect. Nitrogen fertilization altered aboveground dry weight partitioning and these changes affected the hydraulic architecture. Variability in the magnitude of the responses was observed among families. The family that was more negatively affected by N fertilization (L) changed its dry weight partitioning and hydraulic conductance in a way that diminished water delivery to the leaves. In this manner, water potential in the leaves was reduced, stomata were partially closed and growth was reduced. The family that was less affected by N fertilization (H) closed the stomata and changes in the anatomy of the xylem probably assisted with maintaining water delivery to the leaves and sustaining growth. Phosphorus fertilization had a positive effect on growth and the changes in the hydraulic architecture did not lead to a decline in leaf water potential.

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## Conflict of interest

None declared.

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